

Annexins in cancer and autoimmune diseases

B. C. Bastian

Klinik und Poliklinik für Hautkrankheiten, Julius-Maximilians Universität Würzburg, Josef-Schneider-Str. 2, D-97080 Würzburg (Germany)

Abstract. Several annexins have been implicated in the pathogenesis of benign and malignant neoplasms of different origins. In some tumours a suppressive action of annexins has been shown, whereas studies of other tumours indicate an involvement of annexins in tumour progression. In the light of the expression of annexins at distinct episodes of fetal development these observations point towards a functional role of annexins in cellular development and differentiation. This view is supported by data that link certain annexins to distinct pathways of signal transduction. Auto-antibodies against several annexins have been detected in patients with autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis and inflammatory bowel disease. Until now it is unclear whether their presence reflects a relevant pathogenetic mechanism or merely represents an unspecific expression of a raised autoimmunity in these patients.

Key words. Annexins; neoplasms; pathology; autoimmune diseases; auto-antibodies; differentiation.

Expression of annexins in tumours

The finding that several annexins are subject to growth-dependent regulation of expression, together with their participation in signalling pathways and cell-cell adhesion, has awoken interest in their role in the pathogenesis of cancer. Considering the related structural properties of the annexins it is of interest that tumour suppressing and promoting attributes have also been demonstrated for different annexins.

In 1983 Hattori et al. reported that AX-1 can induce differentiation in the human histiocytic lymphoma cell line U937 [1]. In their experiments a similar effect could be obtained by treatment of the cells for 6 days with dexamethasone. This dexamethasone-inducible differentiation could be blocked by a monoclonal anti-AX-1 antibody, suggesting that the dexamethasone effect was mediated by AX-1. However, other investigators failed to confirm AX-1 mRNA and protein induction by dexamethasone in U937 cells [2] as well as in primary human macrophages [3]. That these discrepancies might be a reflection of different culture conditions has been shown by Schlaepfer et al. [4]. The cellular level of AX-1 varies with the growth state of cells, with proliferating cells having significantly higher cellular levels and synthesis rates than quiescent cells. Differentiation induction by AX-1 was also reported for the human lung cell adenocarcinoma cell line A549 [5, 6] and the human squamous cell carcinoma cell line SqCC/Y1 [7]. Immunohistochemical analyses have revealed increased

expression of AX-1 in a variety of central and peripheral nervous system tumours [8] and squamous cell carcinoma of the skin [9].

AX-2 has also been shown to underlie a growth-dependent regulation and to be inducible by mitogenic substances [10, 11]. In contrast to the described tumour suppressive effects assigned to AX-1 the association of AX-2 phosphorylation with retroviral transformation has raised the suspicion that AX-2 could be involved in the pathogenesis of cancer [12–14]. AX-2 has been shown to be highly abundant in human hepatocellular carcinoma but not in normal liver, human fetal tissue or regenerating rat liver after injury [15]. Overexpression of AX-2 mRNA and protein have further been demonstrated in human pancreatic cancer and pancreatic cancer derived cell lines [16, 17], multi-drug resistant small cell lung cancer [18], and high but not low grade gliomas [19, 20]. In the Eker rat hereditary renal carcinoma model, a dominant disorder with a defect in the rat analogue of the human tuberous sclerosis (*TSC2*) gene, a differential analysis showed the AX-2 heavy chain to be one of four genes with increased expression compared to normal animals [21]. Low baseline AX-2 expressions and strong increases after induction of differentiation are found in the PC12 rat adrenal pheochromocytoma and the F9 murine teratocarcinoma cell lines [22, 23]. Tressler et al. have demonstrated that AX-2 and AX-6 expressed on murine RAW117 lymphoma cells serve as adhesion molecules for tumour cell–endothelial cell binding [24, 25]. They showed that AX-2 and AX-6 are present on the external plasma membrane and that the binding of tumour cells could be significantly reduced by antibodies against AX-2 or AX-6. These findings indicate a pivotal role of certain AXs in cell-cell interaction.

Current address: Cancer Genetics Program, University of California San Francisco, Cancer Center, Box 0808, San Francisco (California 94143-0808, USA), Fax +1 415 476 8218, e-mail: bastian@cc.ucsf.edu

AXs-1, 2, 4, 5 and 6 were also shown to be expressed by cultured osteoblasts and the human osteosarcoma cell line MG-63 [26]. In this study the amount of AX-5 found in MG-63 cells was three times higher than in the primary osteoblast cultures. The authors also noted an influence on AX-5 levels on the state of growth of the culture. Karube et al. found a decrease of AX-5 mRNA and protein in carcinomas of the uterine cervix and endometrium when compared to normal tissues [27]. As AX-5 has been shown to exert an inhibitory action on protein kinase C [28, 29] the decreased levels of AX-5 might lead to a dysregulated activation of protein kinase C.

In contrast to AX-1 and -2 which are regulated during normal cell growth, the expression of AX-6 appears to be constitutive in most cell lines [30]. Tumour suppressive effects have been assigned to AX-6 in certain model systems. AX-6 is not expressed in the human squamous cell carcinoma line A431 which is characterized by a lack of contact inhibition and reduced growth factor requirement. After transfection with the AX-6 gene A431 cells stopped proliferating after reaching confluence [31] and grew smaller tumours than the non-transfected cells in mice [32]. Recently, AX-6 has been demonstrated to be differentially expressed in a murine melanoma cell line when compared to a syngeneic Melan-a-immortalized melanocyte cell line [33].

Acute promyelocytic leukemia (APL) is a disorder characterized by a balanced t(15;17) translocation in which the breakpoint involves the retinoic acid receptor- α and the *PML* gene [34]. AX-8 was shown to be overexpressed in APL cells in the majority of cases [35, 36]. As the AX-8 gene is located on chromosome 10 its overexpression cannot be directly related to the translocation. Interestingly, all-trans-retinoic acid, a strikingly effective drug to induce remissions in APL patients, is able to reduce the AX-8 expression in the APL-derived cell line NB4 [35]. This has been shown to be due to transcriptional regulation of the AX-8 gene [36]. The negative response of AX-8 expression to all-trans-retinoic acid supports the notion that AX-8 might act as a signal transducer involved in regulation of cell growth and differentiation.

Annexins as potential auto-antigens in autoimmune diseases

Auto-antibodies against AX-1 in patients with rheumatic diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) were first described by Hirata et al. in 1981 [37]. These investigators found that patients' sera were able to abrogate the inhibitory action of a partly purified rabbit lipocortin on phospholipase A₂. This effect was decreased after absorption of the IgM fraction but not the IgG fraction of the sera, which suggests that circulating IgM antibodies

against AX-1 were present in those patients. Later, AX-1 of the IgM and to a lesser extent of the IgG-type were found in patients with RA and SLE using a recombinant human AX-1 and a correlation with disease activity was claimed [38]. Initially, it was suggested that corticosteroids might, by the induction of AX-1, induce the formation of AX-1 auto-antibodies, and that their presence might impair the inhibitory action of corticosteroids on phospholipase A₂ [38]. However, a causal role of corticosteroids in the induction of AX-1 auto-antibodies seems unlikely, because asthma patients treated with corticosteroids do not show AX-1 auto-antibodies [39]. Furthermore, no association of AX-1 auto-antibodies with either serum phospholipase A₂ levels or activity could be shown [40]. Patients with SLE were also reported to have auto-antibodies against AX-5 [41]. In this study the auto-antibodies were more common in patients who additionally had positive anti-cardiolipin antibodies or lupus anticoagulant. AX-1 auto-antibodies have also been demonstrated in patients with inflammatory bowel diseases, such as Crohn's disease or ulcerative colitis, who lack any association with corticosteroid treatment [42]. Interestingly, in this study patients with active confirmed bacterial diarrhoea had the highest titres of AX-1 auto-antibodies, suggesting that their presence might not be confined to autoimmune diseases but might reflect a nonspecific reaction to inflammation. This view is supported by studies demonstrating auto-antibodies against AX-1, AX-2, AX-3, AX-4, AX-5, and AX-6 in a plethora of inflammatory and neoplastic skin diseases without a clear correlation to any disease group including autoimmune diseases [43, 44]. For obvious reasons, a final judgement on the relevance of AX auto-antibodies requires the elucidation of the functions of AXs.

- 1 Hattori T., Hoffman T. and Hirata F. (1983) Differentiation of a histiocytic lymphoma cell line by lipomodulin a phospholipase inhibitory protein. *Biochem. Biophys. Res. Commun.* 111: 551-559
- 2 Isacke C. M., Lindberg R. A. and Hunter T. (1989) Synthesis of p36 and p35 is increased when U-937 cells differentiate in culture but expression is not inducible by glucocorticoids. *Molec. Cell Biol.* 9: 232-240
- 3 Bronnegard M. O., Andersson O., Edwall J., Lund G., Norstedt G. and Carlstedt-Duke J. (1988) Human calpactin II (lipocortin I) messenger ribonucleic acid is not induced by glucocorticoids. *Molec. Endocrinol.* 2: 732-739
- 4 Schlaepfer D. D. and Haigler H. T. (1990) Expression of annexins as a function of cellular growth state. *J. Cell Biol.* 111: 19685-21061
- 5 Croxtall J. D., Waheed S., Choudhury Q., Anand R. and Flower R. J. (1993) N-terminal peptide fragments of lipocortin-1 inhibit A549 cell growth and block EGF-induced stimulation of proliferation. *Int. J. Cancer* 54: 153-158
- 6 Croxtall J. D. and Flower R. J. (1992) Lipocortin 1 mediates dexamethasone-induced growth arrest of the A549 lung adenocarcinoma cell line. *Proc. Natl Acad. Sci. USA* 89: 3571-3575
- 7 Violette S. M., King I., Browning J. L., Pepinsky R. B., Wallner B. P. and Sartorelli A. C. (1990) Role of lipocortin 1 in the glucocorticoid induction of the terminal differentiation of a human squamous carcinoma. *J. Cell Physiol.* 142: 70-77

- 8 Johnson M. D., Kamso-Pratt J., Pepinsky R. B. and Whetsell W. O. J. (1989) Lipocortin-I immunoreactivity in central and peripheral nervous system glial tumours. *Hum. Pathol.* **20**: 772-776
- 9 Bastian B. C., van der Piepen U., Römlsch J., Pâques E. P. and Bröcker E. B. (1993) Localization of annexins in normal and diseased human skin. *J. Dermatol. Sci.* **6**: 225-234
- 10 Chiang Y., Schneiderman M. H. and Vishwanatha J. K. (1993) Annexin II expression is regulated during mammalian cell cycle. *Cancer Res.* **53**: 6017-6021
- 11 Kreutzer J. C. and Hirschhorn R. R. (1990) The growth-regulated gene *1B6* is identified as the heavy chain of calpactin I. *Exp. Cell Res.* **188**: 153-159
- 12 Radke K., Gilmore T. and Martin G. S. (1980) Transformation by Rous sarcoma virus: a cellular substrate for transformation-specific protein phosphorylation contains phosphotyrosine. *Cell* **21**: 821-828
- 13 Erikson E. and Erikson R. L. (1980) Identification of a cellular protein substrate phosphorylated by the avian sarcoma virus-transforming gene product. *Cell* **21**: 829-836
- 14 Ozaki T. and Sakiyama S. (1993) Molecular cloning of rat calpactin I heavy-chain cDNA whose expression is induced in v-src-transformed rat culture cell lines. *Oncogene* **8**: 1707-1710
- 15 Frohlich M., Motte P., Galvin K., Takahashi H., Wands J. and Ozturk M. (1990) Enhanced expression of the protein kinase substrate p36 in human hepatocellular carcinoma. *Molec. Cell Biol.* **10**: 3216-3223
- 16 Vishwanatha J. K., Chiang Y., Kumble K. D., Hollingsworth M. A. and Pour P. M. (1993) Enhanced expression of annexin II in human pancreatic carcinoma cells and primary pancreatic cancers. *Carcinogenesis* **14**: 2575-2579
- 17 Kumble K. D., Hirota M., Pour P. M. and Vishwanatha J. K. (1992) Enhanced levels of annexins in pancreatic carcinoma cells of Syrian hamsters and their intrapancreatic allografts. *Cancer Res.* **52**: 163-167
- 18 Cole S. P., Pinkoski M. J., Bhardwaj G. and Deeley R. G. (1992) Elevated expression of annexin II (lipocortin II, p36) in a multidrug resistant small cell lung cancer cell line. *Br. J. Cancer* **65**: 498-502
- 19 Reeves S. A., Chavez-Kappel C., Davis R., Rosenblum M. and Israel M. A. (1992) Developmental regulation of annexin II (Lipocortin 2) in human brain and expression in high grade glioma. *Cancer Res.* **52**: 6871-6876
- 20 Roseman B. J., Bollen A., Hsu J., Lamborn K. and Israel M. A. (1994) Annexin II marks astrocytic brain tumours of high histologic grade. *Oncol. Res.* **6**: 561-567
- 21 Hino O., Kobayashi E., Nishizawa M., Kubo Y., Kobayashi T., Hirayama Y. et al. (1995) Renal carcinogenesis in the Eker rat. *J. Cancer Res. Clin. Oncol.* **121**: 602-605
- 22 Fox M. T., Prentice D. A. and Hughes J. P. (1991) Increases in p11 and annexin II proteins correlate with differentiation in the PC12 pheochromocytoma. *Biochem. Biophys. Res. Commun.* **177**: 1188-93
- 23 Harder T., Thiel C. and Gerke V. (1993) Formation of the annexin II/p112 complex upon differentiation of F9 teratocarcinoma cells. *J. Cell Sci.* **104**: 1109-1117
- 24 Tressler R. J., Yeatman T. and Nicolson G. L. (1994) Extracellular annexin VI expression is associated with divalent cation-dependent endothelial cell adhesion of metastatic RAW117 large-cell lymphoma cells. *Exp. Cell Res.* **215**: 395-400
- 25 Tressler R. J., Updyke T. V., Yeatman T. and Nicolson G. L. (1993) Extracellular annexin II is associated with divalent cation-dependent tumour cell-endothelial cell adhesion of metastatic RAW117 large-cell lymphoma cells. *J. Cell Biochem.* **53**: 265-276
- 26 Mohiti J., Walker J. H. and Caswell A. M. (1995) Studies on annexins in primary cultures of human osteoblasts and in the human osteosarcoma cell line MG-63. *Biochem. Soc. Trans.* **23**: 36S
- 27 Karube A., Shidara Y., Hayasaka K., Maki M. and Tanaka T. (1995) Suppression of calphobindin I (CPB I) production in carcinoma of uterine cervix and endometrium. *Gynecol. Oncol.* **58**: 295-300
- 28 Shibata S., Sato H. and Maki M. (1992) Calphobindins (placental annexins) inhibit protein kinase C. *J. Biochem.* **112**: 552-556
- 29 Schlaepfer D. D., Jones J. J. and Haigler H. T. (1992) Inhibition of protein kinase C by annexin V. *Biochemistry* **31**: 1886-1891
- 30 Moss S. E., Jacob S. M., Davies A. A. and Crumpton M. J. (1992) A growth-dependent post-translational modification of annexin VI. *Biochim. Biophys. Acta* **1160**: 120-126
- 31 Theobald J., Smith P. D., Jacob S. M. and Moss S. E. (1994) Expression of annexin VI in A431 carcinoma cells suppresses proliferation: a possible role for annexin VI in cell growth regulation. *Biochim. Biophys. Acta* **1223**: 383-390
- 32 Theobald J., Hanby A., Patel K. and Moss S. E. (1995) Annexin VI has tumour-suppressor activity in human A431 squamous epithelial carcinoma cells. *Br. J. Cancer* **71**: 786-788
- 33 Francia G., Mitchell S. D., Moss S. E., Hanby A. M., Marshall J. F. and Hart I. R. (1996) Identification by differential display of annexin-VI, a gene differentially expressed during melanoma progression. *Cancer Res.* **56**: 3855-3858
- 34 Warrell R. P., de Thé H., Wang Z. Y. and Degos L. (1993) Acute promyelocytic leukemia. *New Engl. J. Med.* **329**: 177-189
- 35 Chang K. S., Wang G., Freireich E. J., Daly M., Naylor S. L., Trujillo J. M. et al. (1992) Specific expression of the annexin VIII gene in acute promyelocytic leukemia. *Blood* **79**: 1802-1810
- 36 Sarkar A., Yang P., Fan Y. H., Mu Z. M., Hauptmann R., Adolf G. R. et al. (1994) Regulation of the expression of annexin VIII in acute promyelocytic leukemia. *Blood* **84**: 279-286
- 37 Hirata F., del Carmine R., Nelson C. A., Axelrod J., Schiffmann E., Warabi A. et al. (1981) Presence of autoantibody for phospholipase inhibitory protein, lipomodulin, in patients with rheumatic diseases. *Proc. Natl Acad. Sci. USA* **78**: 3190-3194
- 38 Goulding N. J., Podgorski M. R., Hall N. D., Flower R. J., Browning J. L., Pepinsky R. B. et al. (1989) Autoantibodies to recombinant lipocortin-I in rheumatoid arthritis and systemic lupus erythematosus. *Ann. Rheum. Dis.* **48**: 843-850
- 39 Wilkinson J. R., Podgorski M. R., Godolphin J. L., Goulding N. J. and Lee T. H. (1990) Bronchial asthma is not associated with auto-antibodies to lipocortin-I. *Clin. Exp. Allergy* **20**: 189-192
- 40 Pruzanski W., Goulding N. J., Flower R. J., Gladman D. D., Urowitz M. B., Goodman P. J. et al. (1994) Circulating group II phospholipase A2 activity and antilipocortin antibodies in systemic lupus erythematosus. Correlative study with disease activity. *J. Rheumatol.* **21**: 252-257
- 41 Matsuda J., Saitoh N., Gohchi K., Gotoh M. and Tsukamoto M. (1994) Anti-annexin V antibody in systemic lupus erythematosus patients with lupus anticoagulant and/or anticardiolipin antibody. *Am. J. Hematol.* **47**: 56-58
- 42 Stevens T. R., Smith S. F. and Rampton D. S. (1993) Antibodies to human recombinant lipocortin-I in inflammatory bowel disease. *Clin. Sci.* **84**: 381-386
- 43 Kraus M., Römlsch J., Bastian B. C., Pâques E. P. and Hartmann A. A. (1992) Detection of human anti-annexin autoantibodies by enzyme immunoassays. *J. Immunoassay* **13**: 411-439
- 44 Bastian B. C., Nuss B., Römlsch J., Kraus M. and Bröcker E. B. (1994) Autoantibodies to annexins: a diagnostic marker for cutaneous disorders? *J. Dermatol. Sci.* **8**: 194-202